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<b>(54) Title:</b> MONOCLONAL ANTIBODIES SPECIFIC FOR FELINE INFECTIOUS PERITONITIS VIRUS  <b>(57) Abstract</b>  The present invention provides novel hybridoma cell lines which produce novel monoclonal antibodies (MoAbs) which specifically bind epitopes found on a structural protein of feline infectious peritonitis virus (FIPV), exhibit no cross-reactivity with relates coronaviruses, and fail to induce antibody-dependent enhancement of infection. The novel MoAbs produced by the hybridoma cell lines of the invention can be use in assays for the detection of feline infectious peritonitis virus in domestic as well as exotic cats, and for the therapeutic and/or prophylactic treatment of cats against feline infectious peritonitis (FIP) from infection by FIPV.		

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## MONOCLONAL ANTIBODIES SPECIFIC FOR FELINE INFECTIOUS PERITONITIS VIRUS

### FIELD OF THE INVENTION

5 The present invention relates generally to feline coronaviruses. More particularly, the invention relates to novel hybridoma cell lines which produce novel monoclonal antibodies which bind epitopes found on a structural protein of feline infectious peritonitis virus (FIPV).

### BACKGROUND OF THE INVENTION

10 Feline infectious peritonitis virus (FIPV) is a member of the genus **Coronavirus** in the family **Coronaviridae**. Coronaviruses are plus-sense, single-stranded RNA viruses with three major structural proteins, the spike (S), membrane (M) and nucleocapsid (N) proteins. The family of coronaviruses is divided into two distinct antigenic groups. One antigenic group contains mouse hepatitis virus, hemagglutinating encephalomyelitis virus of swine, neonatal calf diarrhea coronavirus, human coronavirus HCV-OC43 and rat coronavirus. The second antigenic group comprises human coronavirus HCV-229E, transmissible gastroenteritis virus (TGEV) of swine, canine coronavirus and FIPV. In addition to these members, a feline enteric coronavirus (FECV) has been isolated, which is closely related to FIPV, TGEV and canine coronavirus.

20 FIPV causes a complex and invariably fatal disease known as feline infectious peritonitis (FIP) in domestic as well as exotic cats. FIP is characterized by anorexia, weight loss and fever. Raised nodules and petechial hemorrhages have been noticed on many internal organs, particularly the spleen and liver of the cat. Infected cats also have atypical serum chemical profiles and hemogram analyses, including high serum aspartate transaminase, serum alanine transaminase and lactate dehydrogenase values as well as hypergammaglobulinemia and other manifestations of immune complex disease.

30 Although natural exposure of cats to FIPV or related coronaviruses appears to occur with a high degree of frequency, only a small percentage of cats develop FIP. The presence of antibodies to FIPV in a large percentage of healthy cats suggests that most natural infections are subclinical. Serologic studies have demonstrated the presence of antibody to FIPV in greater than 80% of cats raised in catteries, but even under these conditions the incidence of disease rarely exceeds 10% of the population. In contrast, experimental infection of specific-pathogen-free cats most often results in death. In spite of its relatively low incidence resulting from natural infection, FIP remains a serious problem for which there is no effective treatment.

- 2 -

Currently available diagnostic assays for FIPV are not specific for FIPV and thus report previous exposure to any of the related coronaviruses. Also, such assays frequently report the production of significant non-specific background. U.S. Patent 4,806,467 to Porter et al., discloses a competitive enzyme-linked immunoabsorbant assay (CELISA) for  
5 detecting antibodies specific to a retrovirus, i.e., equine infectious anemia (EIA). The authors note that a CELISA has been developed for detecting antibodies for FIPV antigens citing U.S. application Serial No. 716,374 filed March 26, 1985, now abandoned, and based on the Fiscus et al., article "Competitive Enzyme Immunoassay for the Rapid  
10 Detection of Antibodies to Feline Infectious Peritonitis Virus Polypeptides", 1985, *J. Clin. Microbiol.*, Vol 22, No. 3, pp.395-401, which discloses monoclonal antibodies specific for viral polypeptides and CELISAs for detection of anti-FIPV antibodies. However, the authors conclude in this article that FIPV and FECV are antigenically indistinguishable.

U.S. Patent 4,774,177 to Marks, discloses enzyme-linked immunoabsorbant assay (ELISA) test kits for detecting FIPV or other coronaviruses. However, Marks does not  
15 employ monoclonal antibodies which specifically recognize only FIPV. The antibodies disclosed by Marks show cross-reactivity with related coronaviruses.

Ingersoll et al., "Comparison of Serologic Assays for Measurement of Antibody Response to Coronavirus in Cats", 1988, *Am. J. Vet. Res.*, Vol 49, No. 9, pp. 1472-1479, discloses a comparison of various assays and their ability to distinguish specific virus  
20 exposures, i.e., FECV and FIPV, in cats. The assays studied include indirect immunofluorescence test (IFT), virus neutralization (VNTN) and enzyme-linked immunoabsorbant assays (ELISAs). The authors concluded that none of the assays were specific enough to discern the specific virus to which the cat had been exposed.

Furthermore, immunity to FIPV is poorly understood and is dependent on factors  
25 seemingly unrelated or indirectly related to antibody titer. Cats with high levels of virus-neutralizing antibody to FIPV frequently develop FIP more rapidly and with more severe clinical signs than do seronegative cats following experimental infection. The unique role of antibody in promoting and exacerbating disease has been documented by passive transfer of FIPV-reactive immune serum or purified immunoglobulin to  
30 seronegative cats. Such antibody-sensitized cats also develop enhanced disease after challenge with FIPV. This phenomenon, referred to as antibody-dependent enhancement (ADE), is believed to occur when virus-antibody immune complexes bind to Fc receptors for immunoglobulin G (FcγR) present on the cell surface of macrophages, the putative target cells of FIPV. Enhanced uptake of virus by macrophages in the presence of  
35 antibody has been demonstrated *in vitro* and is believed to play an important role in the increased virulence and dissemination of FIPV observed in sensitized cats.

- 3 -

Previous studies have shown that the spike protein (S), one of 3 structural proteins of FIPV, is responsible for eliciting virus-neutralizing antibodies. However, when kittens were vaccinated with recombinant vaccinia virus expressing either S, nucleocapsid protein (N), or membrane protein (M), ADE was observed after challenge with FIPV only in those kittens vaccinated with the S recombinant. This suggests that epitopes located on S are responsible for mediating both virus neutralization and ADE. At present, vaccination has been consistently hindered by problems encountered with ADE, and antibody titers have little correlation with protection.

Consequently, it is currently not possible to differentiate FIPV, a serious systemic disease which often results in death of the cat, with antigenically and biologically similar coronaviruses, such as, for example, FECV, which is a subclinical or mild enteric infection in cats.

### SUMMARY OF THE INVENTION

The present invention provides novel hybridoma cell lines which produce novel monoclonal antibodies (MoAbs) which specifically recognize various strains of feline infectious peritonitis virus (FIPV).

More particularly, the present invention provides MoAbs which specifically bind epitopes found on a structural protein of feline infectious peritonitis virus. The MoAbs of the present invention exhibit no cross-reactivity with related coronaviruses, and two of the MoAbs (19E8.4 and 21D10.2) are FIPV neutralizing with low titers (1:10). Furthermore, the MoAbs of the invention fail to induce antibody-dependent enhancement of infection.

The Monoclonal antibodies produced by the hybridoma cell lines of the present invention can be used in assays for the detection of feline infectious peritonitis virus in domestic as well as exotic cats and may be used for the therapeutic and/or prophylactic treatment of cats against feline infectious peritonitis (FIP) from infection by FIPV.

The hybridomas are produced by fusing an immortal cell, a myeloma cell having the ability to replicate indefinitely in cell culture, and an effector immune cell following immunization of the immune cell host with a preparation of a strain of feline infectious peritonitis virus.

While several individual hybridoma cell lines producing monoclonal antibodies which bind epitopes found on the structural proteins of feline infectious peritonitis virus are disclosed herein, the present invention adds to the state of the art an entire family of hybridomas producing monoclonal antibodies specific only to structural protein-associated

- 4 -

epitopes of feline infectious peritonitis virus and exhibit no cross-reactivity with related coronaviruses.

## BRIEF DESCRIPTION OF THE DRAWINGS

5 Fig. 1 is a radioimmunoprecipitation and SDS-polyacrylamide gel electrophoretic analysis of FIPV proteins.

Fig. 2 is a radioimmunoprecipitation and SDS-polyacrylamide gel showing monoclonal antibody specificity for FIPV proteins.

10 Fig. 3 is a radioimmunoprecipitation and SDS-polyacrylamide gel showing denatured FIPV-infected cell lysates with MoAbs.

Figs. 4(a) and 4(b) are immunofluorescence assays of cells expressing recombinant FIPV S protein using S-specific MoAbs or N- and M- specific MoAbs, respectively.

15 FIG. 5 is a schematic representation of the relationship between neutralizing and enhancing MoAbs to the S protein.

## DETAILED DESCRIPTION OF THE INVENTION

20 The present invention involves certain hybrid cells, and their functional equivalents, capable of producing monoclonal antibodies (MoAbs) which recognize (bind) epitopes found on a structural protein of feline infectious peritonitis virus (FIPV). The MoAbs disclosed herein are characterized according to protein specificity (i.e., spike protein (S), nucleocapsid protein (N) and membrane protein (M)), immunoglobulin subclass, virus neutralization, reactivity with related coronaviruses, and ability to induce antibody  
25 dependent enhancement (ADE) of FIPV infection in vitro.

More particularly, hybridoma cell lines 24D11.8, 19E8.4, 23B4.9 and 21D10.2 are provided which produce MoAbs 24D11.8, 19E8.4, 23B4.9 and 21D10.2 which bind epitopes found on the spike protein of feline infectious peritonitis virus. These MoAbs are specific for only FIPV in that they exhibit no cross-reactivity with related coronaviruses.  
30 As used herein, cross-reactivity is defined as the ability of an antiserum or antibody specific for FIPV to react with other coronaviruses, such as, for example, feline enteric coronavirus, canine coronavirus, and porcine transmissible gastroenteritis virus.

In addition, the MoAbs of the present invention were tested for their ability to mediate antibody-dependent enhancement (ADE) in primary cultures of feline peritoneal  
35 macrophages, or in the mouse macrophage cell line IC-21.

- 5 -

Hybridoma formation and monoclonal antibody production may be effected by many different techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal i.e., mouse, which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*.

5 The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown  
10 either *in vivo* or *in vitro* so as to produce large quantities of antibody (for description of the theoretical basis and practical methodology of fusing such cells, see Kohler and Milstein, *Nature* (1975) 256:495, the disclosure of which is hereby incorporated by reference). While such methods are described in further detail hereinafter, it will be appreciated by those skilled in the art that modifications and additions to the techniques  
15 may be made without departing from the scope of the present invention.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (mice) with FIPV. Such immunizations are repeated as necessary at intervals of up to several weeks so as to obtain a sufficient titer of antibodies. The virus is carried in appropriate solutions or adjuvants. Following the last antigen boost, the animals are  
20 sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol (PEG) or other fusing agents (See: Milstein and Kohler, 1976, *Eur. J. Immunol.* Vol 6, p. 511, the disclosure of which is hereby  
25 incorporated by reference). This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

30 Enzyme deficiencies may include, for example, thymidine kinase (TK) or hypoxanthine-guanine phosphoribosyl transferase (HGPRT). These deficiencies allow selection for fused cells according to their ability to grow in, for example, hypoxanthine-aminopterin-thymidine medium (HAT). Preferably, the immortal fusion partners utilized are derived from a line which does not secrete immunoglobulin.

35 Individual fused cells are grown in individual tissue culture wells. Feeder cells, such as irradiated thymocytes or other cells, may be used to increase the viability of the

- 6 -

cells. Hybridoma culture supernatants from the individual wells are assayed for antibody binding to FIPV by suitable detection methods known in the art, such as enzyme-linked immunoassay (EIA) and immunodot assay. The Immunodot method can also be used to screen for clones expressing FIPV antibodies according to the procedures of Towbin et al.,  
5 *Immunol. Method.*, 1984, Vol 72, p. 313, the disclosure of which is hereby incorporated by reference. Additionally, other screening systems known to those in the art can be utilized.

Large quantities of monoclonal antibodies from secreting hybridomas are produced by injecting the clones into the peritoneal cavity of mice and harvesting the ascites fluid therefrom. The mice, preferably primed with pristane or other tumor-promoter and  
10 immunosuppressed chemically or by irradiating, may be of various strains, such as New Zealand Black or Balb/c strains. The ascites fluid is harvested from the mice and the monoclonal antibody purified therefrom, for example, by CM Sepharose column or other chromatographic means. High titers of antibodies may be so recovered. Alternatively, the hybridomas may be cultured *in vitro* in a variety of ways, utilizing either perfusion  
15 cultures or suspension cultures, both in batch or continuous culture processes, and monoclonal antibodies recovered from the culture medium or supernatant.

The monoclonal antibodies of the present invention so produced have a number of diagnostic and therapeutic uses. They can be used as *in vitro* diagnostic agents to test for the presence of FIPV in cats by subjecting cat fluids, secretions, or extracts to  
20 immunoassay protocols. Such assays may include radioimmunoassay, EIA or chemiluminescent format. Alternatively, a competitive immunoassay or a "sandwich" type assay can be employed. Such histochemical methods are well-known in the art; protocols are found, for example, in *Methods in Immunodiagnosis*, 2nd edition, Rose and Bigazzi, eds., John Wiley and Sons, 1980; and in Campbell et al., *Methods of Immunology*, W.A.  
25 Benjamin, Inc., 1964, which references are hereby incorporated by reference.

It is believed that the monoclonal antibodies of the present invention may be used therapeutically to treat FIPV infections. The antibodies can be administered either intravenously or intramuscularly in a physiologically acceptable carrier, either alone or in combination with antibiotics. Although to do so may affect the binding characteristics of  
30 the present monoclonal antibodies, they may be lyophilized for storage and shipment and reconstituted prior to administration.

Additionally, the monoclonal antibodies of the invention can be used prophylactically in cats at risk for FIPV infection. Administration of effective amounts of these monoclonal antibodies serves to enhance the cats's potential ability to defend against  
35 FIPV, thereby lessening the risk of subsequent infection, especially since these antibodies do not induce antibody-dependent enhancement (ADE).

- 7 -

Among the effects of treatment with several of these monoclonal antibodies are the neutralization of the virus, presumably by opsonization and phagocytosis. For all such diagnostic, prophylactic and therapeutic uses, the monoclonal antibodies and other necessary reagents and appropriate devices and accessories can be provided in kit form so as to be readily available and easily used.

The following examples are offered by way of illustration and not limitation.

### EXAMPLE I

#### Materials and Methods

##### 1. Production of Monoclonal Antibodies (MoAbs)

The 79-1146 strain of FIPV as described by Pedersen et al., "Pathogenicity studies of feline coronavirus isolates 79-1146 and 79-1683", 1984, *Am. J. Vet. Res.*, Vol 45, pp. 2580-2585, the disclosure of which is hereby incorporated by reference, was used to immunize Balb/c mice (Charles Rivers, Wilmington, Mass.) for the production of antibody-secreting lymphocytes to be fused with the murine myeloma cell line Sp2/O-Ag14 as described by Schulman et al., "A better cell line for making hybridomas secreting specific antibodies", 1978, *Nature*, Vol 276, pp. 269-270; and by Harlow et al., "Antibodies: A Laboratory Manual", pp. 139-281, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., which disclosures are hereby incorporated by reference. Crandell feline kidney (CRFK) cells (American Type Culture Collection, Rockville, MD) were infected with FIPV, and virus was harvested at 36 hours after infection. Virus was frozen at -70°C and clarified by centrifugation for 15 minutes at 800 xg. The virus-containing supernatant was concentrated approximately ten-fold in a tangential flow ultrafiltration device (Minitan Ultrafiltration System, Millipore Corporation, Bedford, MA) fitted with a 100,000 molecular weight cutoff membrane as described by Mathes et al., "Purification of feline leukemia virus from large volumes of tissue culture fluid", 1977, *J. Clin. Micro.*, Vol 5, pp. 372-374, which disclosure is hereby incorporated by reference. Concentrated virus was layered over a cushion of 35% (w/w) sucrose in TEN buffer (10 mM Tris pH 7.5, 1mM EDTA, 100 mM NaCl) and pelleted by centrifugation for 3 hours at 27,000 xg in a SW28 rotor at 4°C. Pelleted virus was resuspended in a small volume of TEN buffer and frozen at -70°C until needed. This material had a titer of about  $10^{7.8}$  plaque forming units/ml and was used without further concentration.

Female, 6- to 8-week old mice (Charles Rivers, Wilmington, Mass.) were inoculated intraperitoneally with 400 µl of a 50:50 mixture of concentrated virus and Freund's complete adjuvant. Mice were boosted 3 times with the same preparation in

- 8 -

Freund's incomplete adjuvant at 2 week intervals, followed by a final intrasplenic immunization with 50 µl of virus 3 days prior to fusion with mouse myeloma cells. The fusion was performed with spleen cells obtained from a mouse with a serum neutralization titer of 1,280 when measured against 100 50% tissue culture infective doses (100 TCID<sub>50</sub>) of FIPV.

Hybridoma colonies secreting antibody to FIPV were cloned by limiting dilution. Ascites fluid was produced by intraperitoneal injection of approximately 10<sup>7</sup> cells from each positive clone 24 hours after intraperitoneal injection of 0.5 ml of Freund's incomplete adjuvant to enhance tumor formation as described by Mueller et al, "Monoclonal antibody production by hybridoma growth in Freund's adjuvant primed mice", 1986, *J. Immunol. Methods*, Vol 87, pp. 193-196. A total of 54 FIPV-specific hybridoma clones and ascites fluid were obtained from this single fusion.

## 2. Cell cultures and viruses

Hybridoma cells were cultured in Dulbecco modified Eagle medium supplemented with 20% fetal bovine serum (FBS), 100 IU of penicillin per ml, 100 µg of streptomycin per ml, 2.5 µg of amphotericin B per ml (Fungizone; E. R. Squibb & Sons, Princeton, NJ), 200 µM glutamine, 10 mM minimal essential medium nonessential amino acids, 100 mM sodium pyruvate, 40 µM 2-mercaptoethanol, 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10<sup>-4</sup> M hypoxanthine, and 1.6 X 10<sup>-5</sup> M thymidine. Type strains of FIPV used in the study included 79-1146 (as described elsewhere herein); UCD1 as described by Pedersen, N.C., "Morphologic and physical characteristics of feline infectious peritonitis virus and its growth in autochthonous peritoneal cell cultures", 1976, *Am. J. Vet. Res.*, Vol 36, pp. 566-572 and Pedersen et al., "Infection studies in kittens utilizing feline infectious peritonitis virus propagated in cell culture", 1982, *Am. J. Vet. Res.*, Vol 42, pp. 363-367, which disclosures are hereby incorporated by reference; DF2 as described by Evermann et al., "Characterization of a feline infectious peritonitis virus isolate", 1981, *Vet. Pathol.*, Vol 18, pp. 256-265, which disclosure is hereby incorporated by reference; and CU1 as described by Baines, J. D., and F. W. Scott, unpublished. Other related coronaviruses used were feline enteric coronavirus 1683 as described by Pedersen et al., "Pathogenicity studies of feline coronavirus isolates 79-1146 and 79-1683", 1984, *Am. J. Vet. Res.*, Vol 45, pp. 2580-2585, the disclosure of which is hereby incorporated by reference; canine coronavirus strains I-71 as described by Binn et al., "Recovery and characterization of a coronavirus from military dogs with diarrhea", 1974, Proceedings 78th Ann. Meeting U.S. Animal Health Assoc., pp. 359-366, which disclosure is hereby incorporated by reference; K378, S378, A76-5 as described by Carmichael, L. E., and M.

- 9 -

J. Appel, James A. Baker Institute for Animal Health, Cornell University, Ithica, N.Y., the disclosure of which is hereby incorporated by reference; and the Miller strain of porcine transmissible gastroenteritis virus as described by Bohl et al., "Antibody responses in serum, colostrum and milk of swine after infection or vaccination with transmissible gastroenteritis virus", 1972, *Infect. Immun.*, Vol 6, pp. 289-301, the disclosure of which is hereby incorporated by reference. All viruses except A76-5 and Miller were grown in CRFK cells cultured in Eagle minimum essential medium supplemented with 10% FBS, 200 IU of penicillin per ml, 100 µg of streptomycin per ml, and 2.5 µg of amphotericin B. The A76-5 and Miller strains were grown in A72 cells cultured in Leibovitz's L-15 medium as described by Olsen et al., "Monoclonal antibodies to the spike protein of feline infectious peritonitis virus mediate antibody-dependent enhancement of infection of feline macrophages", 1992, *J. Virol.*, Vol 66; and Stoddart et al., "Isolation and identification of feline peritoneal macrophages for *in vitro* studies of coronavirus-macrophage interactions", 1988, *J. Leukoc. Biol.*, Vol 44, pp. 319-328, which disclosures are hereby incorporated by reference. A recombinant pox virus containing full length S gene of FIPV-79-1146 (rPV-S) and expressing S in cell culture as described by De Groot et al., "cDNA cloning and sequence analysis of the gene encoding the peplomer protein of feline infectious peritonitis virus", 1987, *J. Gen. Virol.*, Vol. 68, pp. 2639-2646; De Groot et al., "Stably expressed FIPV peplomer protein induces cell fusion and elicits neutralizing antibodies in mice", 1989, *Virology*, Vol. 171, pp. 493-502; Olsen et al., "Monoclonal antibodies to the spike protein of feline infectious peritonitis virus mediate antibody-dependent enhancement of infection of feline macrophages", 1992, *J. Virol.*, Vol. 66; and Vennema et al., "Early death after feline infectious peritonitis virus challenge due to recombinant vaccinia virus immunization", 1990, *J. Virol.*, Vol. 64, pp. 1407-1409, which disclosures are hereby incorporated by reference, was grown in Vero cells. Vero cells were cultured in Eagle minimum essential medium, and the mouse macrophage cell line IC-21 (American Type Culture Collection) was cultured in RPMI 1640 medium, with 10% FBS and antibiotics. Primary cultures of feline peritoneal macrophages were collected from a single SPF cat and cultured in Leibovitz's L-15 medium, as elsewhere described herein. All cell lines and FBS were determined to be free of mycoplasma and noncytopathic bovine viral diarrhea virus.

### 3. Immunofluorescence procedures

Hybridoma colonies were screened based on the reactivity of their culture supernatants in an indirect immunofluorescence assay (IFA) with FIPV-infected CRFK cells. Infected cells were first transferred to multispot Teflon-coated microscope slides

- 10 -

(Cel-Line Associates, Newfield, NH) 15 hours after infection and incubated at 37°C for an additional 3 hours. Cells were fixed in 100% acetone, and slides were kept at -70°C until used. When hybridoma colonies were ready for screening, a small amount of culture supernatant was added to wells of the prepared slides and incubated for 30 minutes at 37°C. After washing in phosphate-buffered saline solution (PBSS), the presence of FIPV-specific antibody was detected using fluorescein-conjugated goat anti-mouse immunoglobulin (Cappel Research Products, Durham, NC) in a second 30 minutes incubation at 37°C. Cells were washed and counterstained with a 0.01% solution of Evans Blue. Uninfected CRFK cells were used as a negative control. Only those hybridoma colonies showing a complete lack of reactivity with uninfected cells were selected for cloning. The reactivity of the MoAbs with the various coronaviruses was determined in a similar fashion, using a 1:200 dilution of ascites fluid and CRFK or A72 cells infected with each of the strains of coronavirus. For IFA with rPV-S, Vero cells were grown in 8-chamber slides (Lab Tek, Nunc, Inc., Naperville, IL) and infected with ten-fold dilutions of rPV-S when confluent. Cells were washed and fixed in acetone when small discrete plaques were visible.

## EXAMPLE II

### Virus Neutralization

20

Samples of ascites fluid were heat-inactivated at 56°C for 30 minutes. Serial two-fold dilutions of MoAb were made in Eagle minimum essential medium containing 10% FBS and antibiotics in 96-well, flat-bottom cell culture plates, retaining 50 µl of each dilution in 2 wells. An equal volume of medium containing 100 TCID<sub>50</sub> of FIPV-79-1146 was added to each well. The plates were incubated at 22°C for 1 hour followed by the addition of approximately  $2 \times 10^4$  CRFK cells per well in complete medium. Plates were incubated at 37°C for 4 days, and the neutralization titers were recorded as the reciprocal of the highest dilution of MoAb that completely inhibited cytopathology.

30

## EXAMPLE III

### Determination of Immunoglobulin Subclass

The immunoglobulin subclass of each MoAb was determined using two commercially available kits (Calbiochem, La Jolla, CA; Zymed Laboratories, San Francisco, CA). Because all MoAbs did not react well by enzyme-linked immunoabsorbant assay, a modified IFA was employed using the same reagents. Fixed

- 11 -

FIPV-infected CRFK cells on multispot slides were incubated with each MoAb, followed by an incubation with rabbit anti-mouse subclass-specific immunoglobulin and a final incubation with fluorescein-conjugated goat anti-rabbit IgG (Zymed Laboratories).

5

#### EXAMPLE IV

##### Radioimmunoprecipitation and Electrophoresis

CRFK cells were infected with FIPV-79-1146 and incubated overnight at 37°C. At 15 hours after infection, cells were incubated for 30 minutes in methionine-free medium without FBS, followed by a 3 hours incubation in medium containing 500 µCi of [35S]methionine and [35S]cysteine per ml (Tran 35S-label, ICN Radiochemicals, Irvine, CA), 1% FBS, and 0.1 times unlabeled methionine. At 18 hours after infection, radiolabeled cells were washed twice with PBSS and lysed in cold lysis buffer (50 mM Tris, pH 8.0, 100 mM NaCl; 5 mM EDTA, 1% Triton X-100, 1% deoxycholic acid, 0.1% sodium dodecyl sulfate (SDS), and 50 µM phenylmethylsulfonyl fluoride). The cell lysate was centrifuged at 10,000 xg for 3 minutes, and the supernatant was stored at -70°C until used.

To determine viral protein specificity, samples of radiolabeled cell lysate containing 1 X 10<sup>6</sup> cpm in 200 µl were combined with 10 µl of a 1:10 dilution of each MoAb and incubated overnight at 4°C. For positive controls, 10 µl of undiluted FIPV-specific feline serum taken from an SPF cat infected with FIPV-79-1146 was used. Negative controls consisted of similar preparations using uninfected cell lysate. Immune complexes were adsorbed by adding either 25 µl of a 50% solution of recombinant protein G bound to Sepharose 4B (Zymed Laboratories) to samples containing MoAb, or 25 µl of recombinant protein A to samples containing feline serum (Boehringer Mannheim Biochemicals, Indianapolis, IN). This was followed by a 1 hour incubation at 22°C on a rocking platform.

Immunoprecipitated proteins were gently pelleted for 30 seconds and washed twice in lysis buffer, resuspended in sample buffer (0.2 M Tris, pH 8.8, 2% SDS, 4 mM dithiothreitol, 5 mM EDTA, 15% glycerol, and 0.01% bromophenol blue), and boiled for 4 minutes. After cooling, iodoacetamide was added to a final concentration of 0.1 M. Samples were incubated for 20 minutes at 22°C and then centrifuged at 10,000 xg for 3 minutes. The supernatants were placed in 12.5% polyacrylamide gels containing 0.1% SDS and subjected to electrophoresis in a mini-gel apparatus (Bio-Rad, Richmond, CA) for 90 minutes at 125 V, using a discontinuous buffer system. Following electrophoresis, gels were subjected to fluorography and autoradiography.

- 12 -

**EXAMPLE V**  
**Production of Neutralization-Resistant**  
**Escape Mutants of FIPV**

5 Neutralizing MoAbs were used to select for FIPV mutants that were resistant to neutralization by the selecting MoAb. One-tenth milliliter of medium containing  $10^3$  TCID<sub>50</sub> of FIPV was added to a 1:10 dilution of neutralizing MoAb to a final volume of 1 ml. The mixture was incubated at 22°C for 1 hour and used to infect CRFK cells in 25 cm<sup>2</sup> tissue culture flasks. After a 1 hour incubation at 37°C, medium was added that  
10 contained a 1:100 dilution of the selecting MoAb. Cultures were incubated for 3 to 7 days until visible FIPV-induced cytopathology was present. After a cycle of freezing and thawing, the selection procedure was repeated a second time with the same MoAb. Mutants were then biologically cloned in 6-well tissue culture plates using a 1:100 dilution of the selecting MoAb in the agarose overlay. Monoclonal antibodies were tested for their  
15 ability to recognize cloned mutants by IFA. Those MoAbs that had previously demonstrated antibody dependent enhancement (ADE) with wild-type FIPV were tested for their ability to induce ADE with the mutants.

**EXAMPLE VI**  
**In Vitro Assay for Antibody Dependent Enhancement (ADE)**

20 All MoAbs were assayed for their ability to induce ADE in primary cultures of feline peritoneal macrophages as elsewhere described herein. Harvested macrophages were grown in 8-chamber slides and used at approximately 24 hours after seeding. MoAbs were kept at 56°C for 30 minutes prior to use to eliminate complement activity. A 1:3,000  
25 dilution of MoAb was mixed with an equal volume of medium containing  $2 \times 10^5$  TCID<sub>50</sub> of FIPV or FIPV-escape mutant and incubated at 37°C for 30 minutes. Macrophage cultures were infected with this mixture and incubated at 37°C for 10.5 hours. Cultures were washed in PBSS, fixed in acetone and stored at -70°C until used. A similar  
30 procedure was used with the mouse macrophage cell line IC-21. Evidence of ADE was determined quantitatively by increased number of FIPV-infected macrophages when compared with macrophages infected with virus in the absence of MoAb. Virus-infected cells were detected by IFA using FIPV-specific feline serum, followed by fluorescein-conjugated goat anti-cat IgG (Cappel Research Products).

35

## EXAMPLE VII

### Recognition Of FIPV Proteins

With reference to Figure 1, there is shown a radioimmunoprecipitation and SDS-polyacrylamide gel electrophoretic analysis of FIPV proteins. CRFK cells were infected with FIPV strain 79-1146 and radiolabeled with [<sup>35</sup>S]methionine and [<sup>35</sup>S]cysteine. Viral-induced proteins were immunoprecipitated from infected (lanes 2 and 4) and mock-infected (lanes 1 and 3) cell lysates, using different feline antisera followed by protein A. The 3 structural proteins of FIPV detected by radioimmunoprecipitation with FIPV-specific antiserum had molecular weights of 205 kilodaltons (kDa) (S), 45 kDa (N), and 26 to 28 kDa (M). Endo- $\beta$ -N-acetylglucosaminidase H treatment of FIPV proteins showed that the doublet seen at 26 and 28 kDa represents the non-glycosylated and glycosylated forms of M, respectively.

A total of 54 MoAbs were tested by radioimmuno-precipitation for their viral-protein specificity, using FIPV-79-1146 infected cell lysates. With reference to Figure 2, radioimmunoprecipitation analysis of MoAb specificity for FIPV proteins is shown. Viral proteins from FIPV-79-1146 cell lysates were immunoprecipitated with feline antiserum (lane 1) or with MoAbs specific for S (lanes 9 and 10), N (lanes 7 and 12) or M (lanes 2 and 8). 47 MoAbs recognized S, 3 MoAbs recognized N, and 4 MoAbs recognized M. Many of the S-specific MoAbs also showed nonspecific reactivity with M, as shown in lanes 9 and 10. When cell lysates were denatured prior to the addition of S-specific MoAbs, the reactivity with M was completely removed. This was accomplished by denaturing concentrated cell lysates with 4 mM dithiothreitol and 2% SDS before diluting samples and adding MoAbs.

Monoclonal antibodies were also tested for their ability to recognize S produced in Vero cells infected with rPV-S by indirect immunofluorescence assay (IFA). With reference to Figures 4(a) and 4(b), IFA of recombinant FIPV S protein is shown. Vero cells were infected with recombinant poxvirus expressing full-length S protein (rPV-S) and processed for IFA using either S-specific MoAbs or N- and M- specific MoAbs. When S-specific MoAbs were used, strong immunofluorescence was observed in localized areas surrounding the focal plaques produced by the pox virus (Fig. 4A). Expression of S was indicated by the presence of large brightly staining multinucleated syncytia in these areas. When N- or M-specific MoAbs were used, both the syncytia and surrounding infected cells were negative (Fig. 4B).

- 14 -

**EXAMPLE VIII**  
**Virus Neutralization And MoAb**  
**Reactivity With Escape Mutants**

5 All MoAbs were tested for their ability to neutralize virus in a standard virus neutralization assay. Nineteen of the 54 MoAbs neutralized FIPV with titers that ranged from 10 to 2,560 as shown in Table 1. The 19 virus-neutralizing MoAbs were all found to be specific for S by radioimmunoprecipitation and strong reactivity with rPV-S.

Each of the neutralizing MoAbs was used to select for neutralization-resistant  
 10 escape mutants of FIPV in order to assess the degree of overlap between the different epitopes involved in neutralization. Monoclonal antibodies that lost their ability to react with a particular escape mutant were grouped together with the MoAb that selected for it. This was based on the belief that loss of reactivity of multiple MoAbs to a single escape mutant was due to recognition of the same neutralizing site. Grouping of the MoAbs in  
 15 this manner indicated that the 19 neutralizing MoAbs were recognizing epitopes located within a maximum of 5 neutralizing sites on S. The 5 groups of neutralizing MoAbs are delineated in Table 1:

20 **TABLE 1**

MoAb	Neutralization Group	Neutralization Titer	Protein Specificity
18A7.4	1	5,120	S
17E1.6	1	32	S
23F4.5	2	20,480	S
18A9.4	3	128	S
30 20C6.10	3	15	S
21F1.8	3	10	S
23A1.8	3	10	S
17D7.4	3	48	S
17D12.4	3	32	S
35 22A12.10	3	30	S
20F3.1	3	12	S
20F11.8	3	24	S
18F2.11	3	16	S
19G11.10	3	64	S
40 21D11.8	3	20	S
23F8.1	4	1,920	S
24H5.4	4	96	S
21D10.2	5	10	S
19E8.4	5	12	S

45

- 15 -

### EXAMPLE IX

#### Reactivity With Coronavirus Strains

The MoAbs were tested for their ability to recognize 4 strains of FIPV, 1 strain of feline enteric coronavirus, 4 strains of canine coronavirus, and 1 strain of transmissible gastroenteritis virus by IFA. While strong cross-reactivity to related coronaviruses was seen with some MoAbs, there was considerable variation in the pattern of reactivity between MoAbs. The greatest degree of variation in reactivity patterns was seen among MoAbs recognizing S as shown in Table 2. S-specific MoAbs recognizing 2 of the 5 neutralizing sites were further divided into subgroups based on their differing reactivity patterns with the different coronaviruses. Most importantly, subgroup 5 of MoAbs (Table 2) failed to react with related coronaviruses.

TABLE 2

15		FIPV				FECV	CCV				TGEV
		1146	UCD1	CU1	DF2	1683	K378	S378	1-71	A76-5	Miller
20	1	+	+	+	+	+	+	+	+	-	+
	2	+	+	+	+	+	+	+	+	-	+
	3.1	+	+	+	+	+/-	+	+	+	-	+
	3.2	+		++	+	+	+/-	+/-	-	-	+
	3.3	+	+	+	+	+	+/-	-	-	-	+
25	3.4	+	+	+	+	+	-	-	-	-	+
	4	+	+	+	+	+	+	+	+	-	+
30	5.1	+	+	+	+	-	-	-	-	-	-
	5.2	+	+	+	+	-	-	-	-	-	-
Reactivity in IFA: -, negative; +/-, moderate; +, strong											

### EXAMPLE X

#### Induction Of ADE In Vitro

All 54 MoAbs were tested individually for their ability to mediate ADE in primary cultures of feline peritoneal macrophages. The results of a similar study using a subset of these MoAbs at multiple dilutions is described elsewhere herein. Antibody-dependent enhancement was seen with 17 of the 47 S-specific MoAbs, but not with MoAbs recognizing M or N, as shown in Table 3. All but 2 of the enhancing MoAbs (24H6.8 and 18H9.1) were also virus neutralizing. Monoclonal antibodies taken from 4 of the 5

- 16 -

neutralizing groups showed ADE, as did a single non-neutralizing MoAb (18H9.1) from and unrelated group. Some variation in the degree of enhancement was seen between MoAbs. Macrophages were harvested by peritoneal lavage and incubated in 8-chamber slides at 37 C. Cells were infected with the UCD1 strain of FIPV at 24 hours after

5 seeding. At 10.5 hours after infection, cells were fixed and processed for IFA. Cells were infected with either virus alone or virus in combination with enhancing and neutralizing MoAb 18A7.4. While no consistent pattern was detected, MoAbs that had the highest neutralizing titers tended to show a greater degree of enhancement.

When the IC-21 cell line of mouse macrophages was tested for susceptibility to  
10 infection with FIPV, there was a complete absence of any evidence of viral infectivity. Macrophages were grown in 8-chamber slides and infected with the UCD1 strain of FIPV. Cells were fixed and processed for IFA at 10.5 hours after infection. Cells were infected with either virus alone, virus in combination with enhancing and neutralizing MoAb 23F8.1. When enhancing MoAbs were combined with virus before infection,  
15 approximately 5% of the cells became infected with FIPV. This was most noticeable using MoAbs that had previously demonstrated a high level of ADE in feline macrophages. Occasional syncytia of FIPV-infected IC-21 cells was noted.

Monoclonal antibodies that demonstrated ADE with wild-type FIPV were tested for their ability to induce ADE with neutralization-resistant escape mutants of FIPV.

20 Individual escape mutants were used to infect CRFK cells in combination with the homologous MoAb that had selected for the mutant or with heterologous MoAbs from different neutralizing groups. Antibody-dependent enhancement was only seen when heterologous MoAbs were used.

- 17 -

TABLE 3

Group	MAb	Enhancement	Neutralization	Subclass
5	24H6.8	+	-	$\gamma$ 2a
	18A7.4	+	+	$\gamma$ 2a
	17E1.6	+	+	$\gamma$ 2a
10	23F4.5	+	+	$\gamma$ 2a
	18A9.4	+	+	$\gamma$ 2a
	20C6.10	+	+	$\gamma$ 2a
15	21F1.8	+	+	$\gamma$ 2a
	23A1.8	+	+	$\gamma$ 2a
	17D7.4	+	+	$\gamma$ 2a
	17D12.4	+	+	$\gamma$ 2a
	22A12.10	-	+	$\gamma$ 1
20	20F3.1	+	+	$\gamma$ 2a
	20F11.8	+	+	$\gamma$ 2a
25	18F2.11	+	+	$\gamma$ 2a
	19G11.10	+	+	$\gamma$ 2a
	21D11.8	+	+	$\gamma$ 2a
30	23F8.1	+	+	$\gamma$ 2a
	24H5.4	-	+	$\gamma$ 1
	21D10.2	-	+	$\gamma$ 1
35	19E8.4	-	+	$\gamma$ 1
	18H9.1	+	-	$\gamma$ 1

## EXAMPLE XI

## Immunoglobulin subclass

Among the 54 MoAbs, all 4 subclasses of mouse immunoglobulin G were represented. There were 17- $\gamma$ 1, 30- $\gamma$ 2a, 3- $\gamma$ 2b, and 4- $\gamma$ 3 MoAbs, all of which were comprised of  $\kappa$  light chains. Monoclonal antibodies that demonstrated either ADE or neutralization were most often of the  $\gamma$ 2a subclass as shown in Table 3. Fifteen of 17 enhancing MoAbs and 14 of 19 neutralizing MoAbs belonged to the  $\gamma$ 2a subclass. However, when both ADE and neutralization were examined together with immunoglobulin subclass, a conspicuous relationship was detected. While all but 2 of the enhancing MoAbs belonged to the  $\gamma$ 2a subclass, the 4 MoAbs that were neutralizing but not enhancing were all of the  $\gamma$ 1 subclass. The apparent  $\gamma$ 1 subclass restriction of these 4

- 18 -

MoAbs were particularly noticeable with MoAb 22A12.10, a member of the larger group of 12 related MoAbs in Group 3. In contrast to the  $\gamma$ 2a subclass of the other 11 enhancing MoAbs in Group 3, MoAb 22A12.10 was the only member that belonged to the  $\gamma$ 1 subclass. A similar difference in subclass in combination with absence of ADE was seen with MoAb 24H5.4 when compared with the related Group-4 MoAb 23F8.1. Only 1 MoAb of the  $\gamma$ 1 subclass (18H9.1) was enhancing, although it differed from the other 4  $\gamma$ 1 MoAbs in that it was non-neutralizing. A brief schematic map of enhancing and neutralizing sites on S and their relationship with immunoglobulin subclass is shown in Figure 5.

### Results

54 MoAbs to FIPV were characterized and used to assess their ability to induce virus neutralization and ADE in cell culture. Only S-specific MoAbs were capable of mediating neutralization or ADE. In many instances individual S-specific MoAbs could mediate both processes (Table 3). Fifteen of the 19 neutralizing MoAbs induced ADE, while only 2 of the 28 non-neutralizing MoAbs recognizing S induced ADE.

Monoclonal antibody reactivity patterns with various coronaviruses and escape mutants revealed that there are at least 5 separate neutralizing sites on S represented by the 19 neutralizing MoAbs. MoAbs recognizing 4 of the 5 sites were capable of inducing enhancement, and neutralization-resistant escape mutants selected with MoAbs from 1 group were still enhanced by MoAbs from another group. We believe that this provides evidence that distinct enhancing sites on S exist which largely correspond to the neutralizing sites on the same protein.

While most of the neutralizing and enhancing epitopes appear to be identical, there were neutralizing MoAbs (Table 3) that did not induce enhancement. A comparison of their immunoglobulin subclass revealed that all of these MoAbs belonged to the  $\gamma$ 1 subclass and differed from the majority of enhancing MoAbs which belonged to the  $\gamma$ 2a subclass.

It is believed that the subclass of the MoAb may play a role in determining whether enhancement occurs, in addition to the apparent requirement for recognition of a neutralizing epitope. The strongest support for this is the lack of enhancing activity with neutralizing MoAb 22A12.10 ( $\gamma$ 1 subclass), when compared with the enhancing activity of 11 related neutralizing MoAbs ( $\gamma$ 2a subclass) in Group 3 (Table 3). One MoAb (18H9.1) of the  $\gamma$ 1 subclass had enhancing activity, indicating that any subclass restrictions related to ADE are not absolute.

- 19 -

A model that attributes differences in ADE to immunoglobulin subclass can be supported by known differences in binding specificities of the different classes of Fc and mice, designated Fc $\gamma$ RI, Fc $\gamma$ RII, and Fc $\gamma$ RIII. The classification is based in part on their differing binding specificities for different subclasses of human and mouse

5 immunoglobulin. With respect to murine IgG subclasses, Fc $\gamma$ RI has the highest binding specificity for  $\gamma$ 2a and  $\gamma$ 3, while Fc $\gamma$ RII primarily binds  $\gamma$ 1 and  $\gamma$ 2b. Fc $\gamma$ RIII binds 3,  $\gamma$ 2a, and  $\gamma$ 2b with decreasing efficiencies, respectively. A disproportionate number of Fc $\gamma$ RI or Fc $\gamma$ RIII receptors on feline peritoneal macrophages could explain the high proportion of  $\gamma$ 2a MoAbs inducing ADE and the almost complete absence of ADE with  
10  $\gamma$ 1 MoAbs. The precise mechanisms responsible for the increased uptake of FIPV by Fc $\gamma$ R-bearing macrophages is not known.

It seems unlikely, however, that the enhancement seen with FIPV is due only to a greater efficiency of virus interaction with its receptor. The sheer magnitude of the increase in numbers of infected cells suggests an alternate pathway. In some instances, the  
15 number of infected cells increased from  $\leq 5\%$  to nearly 100% in the presence of antibody. Additionally, we were able to infect a low percentage of mouse macrophages with FIPV in the presence of antibody, even though these cells appear to lack any receptor for FIPV. It seems more likely that an alternative, yet highly efficient form of Fc $\gamma$ R-mediated endocytosis is occurring. Nevertheless, the close association of ADE with virus  
20 neutralization and the observed functional differences in immunoglobulin subclass should further extend our understanding of the basic pathogenesis of FIP.

The present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, however, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

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- 20 -

**WE CLAIM:**

1. A monoclonal antibody, or reactive fragment thereof, which binds an epitope found on a structural protein of feline infectious peritonitis virus, said antibody  
5 exhibiting no cross-reactivity with related coronaviruses.
2. The antibody of claim 1, wherein said structural protein is selected from the group consisting of spike protein, nucleocapsid protein and membrane protein
- 10 3. The antibody of claim 2, wherein said protein is the spike protein.
4. The antibody of claim 1, wherein said feline infectious peritonitis virus is group II virus.
- 15 5. The antibody of claim 1, wherein said antibody is mouse IgG antibody.
6. The antibody of claim 5, wherein said IgG subclass is gamma 1.
7. The antibody of claim 6, wherein said IgG gamma 1 antibody is comprised  
20 of kappa light chains.
8. The antibody of claim 1, wherein said antibody is virus neutralizing.
9. The antibody of claim 1, wherein said antibody fails to induce  
25 antibody-dependent enhancement of feline infectious peritonitis infection.
10. A hybridoma cell line 24D11.8, having A.T.C.C. designation No. \_\_\_\_.
11. A monoclonal antibody, or binding fragment thereof, produced by the cell  
30 line of claim 10.
12. A hybridoma cell line 19E8.4, having A.T.C.C. designation No. \_\_\_\_.
13. A monoclonal antibody, or binding fragment thereof, produced by the cell  
35 line of claim 12.

- 21 -

14. A hybridoma cell line 23B4.9, having A.T.C.C. designation No. \_\_\_\_.
15. A monoclonal antibody, or binding fragment thereof, produced by the cell line of claim 14.
- 5 16. A hybridoma cell line 21D10.2, having A.T.C.C. designation No. \_\_\_\_.
17. A monoclonal antibody, or binding fragment thereof, produced by the cell line of claim 16.
- 10 18. A hybridoma cell line producing the monoclonal antibody, or functional equivalent thereof, according to claim 1.
- 15 19. A method for therapeutically treating a cat against feline infectious peritonitis resulting from infection by feline infectious peritonitis virus, said method comprising administering to a cat a therapeutically effective amount of the monoclonal antibody of claims 1, 11, 13, 15 or 17.
- 20 20. A method for prophylactically treating a cat against feline infectious peritonitis resulting from infection by feline infectious peritonitis virus, said method comprising administering to a cat a prophylactically effective amount of the monoclonal antibody to claims 1, 11, 13, 15 or 17.
- 25 21. A vaccine formulation comprising an immunologically effective amount of the monoclonal antibody of claims 1, 11, 13, 15 or 17, or a fragment or portion thereof, and a physiologically acceptable carrier.

1/5

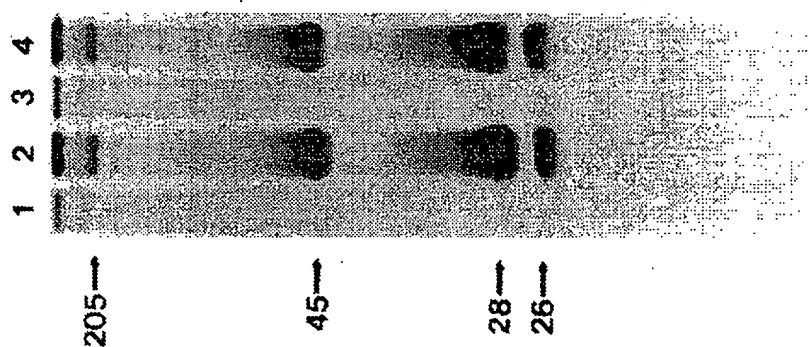


FIG. 1

2/5

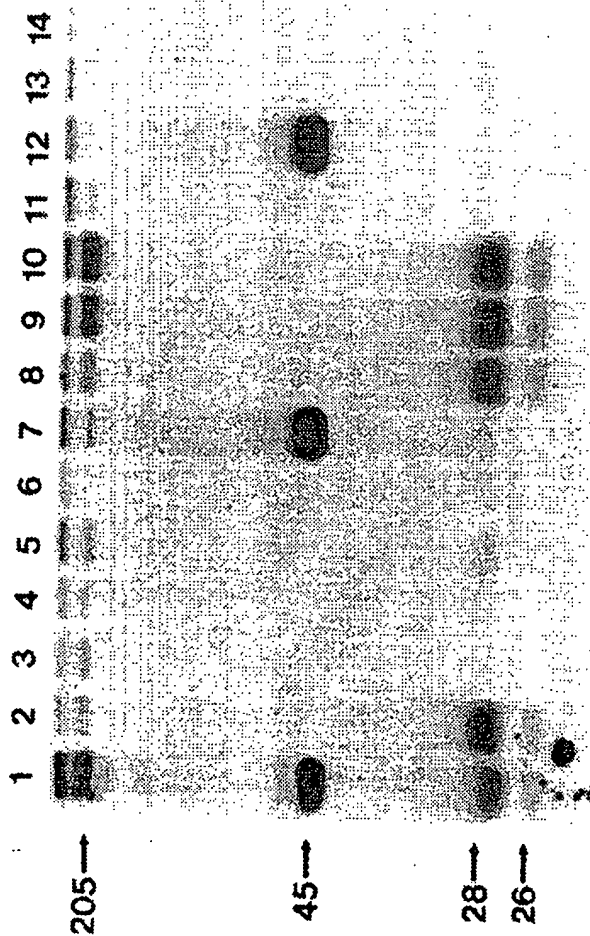
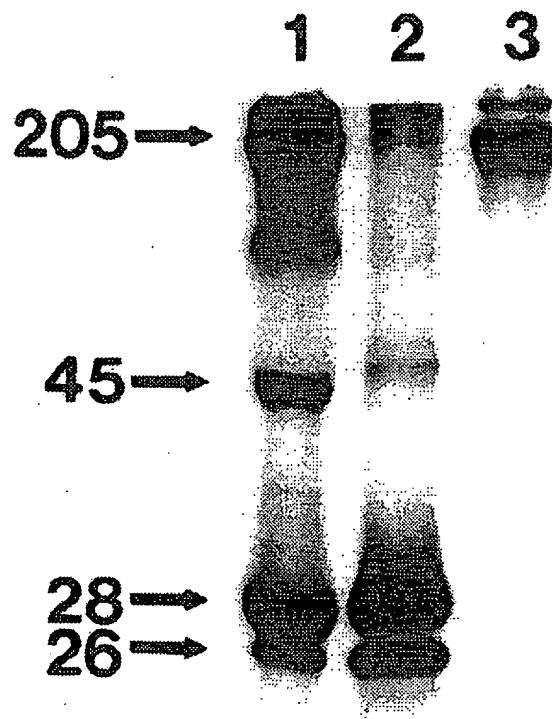


FIG. 2

3/5

FIG. 3

4/5

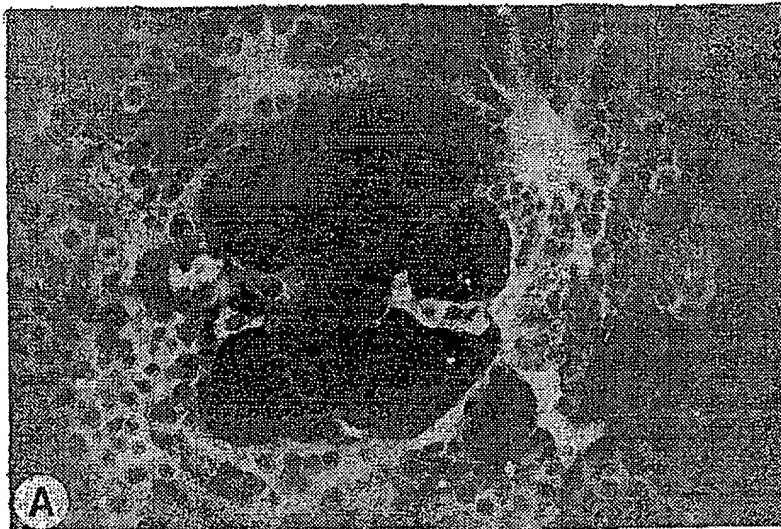


FIG. 4A

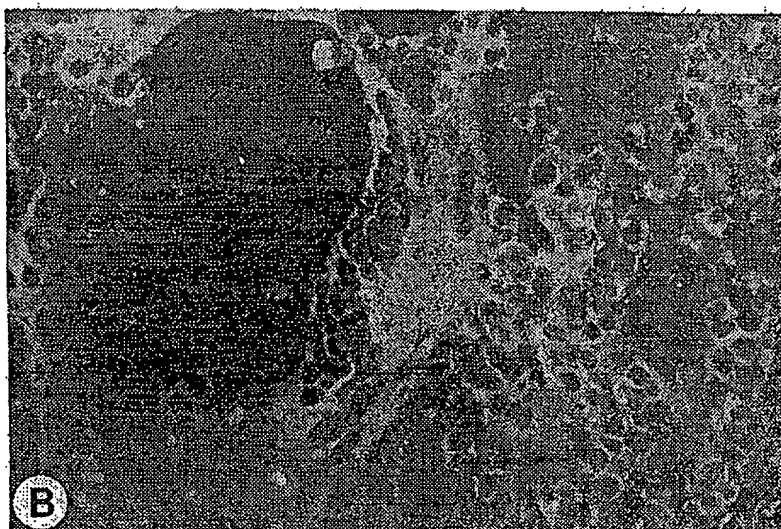
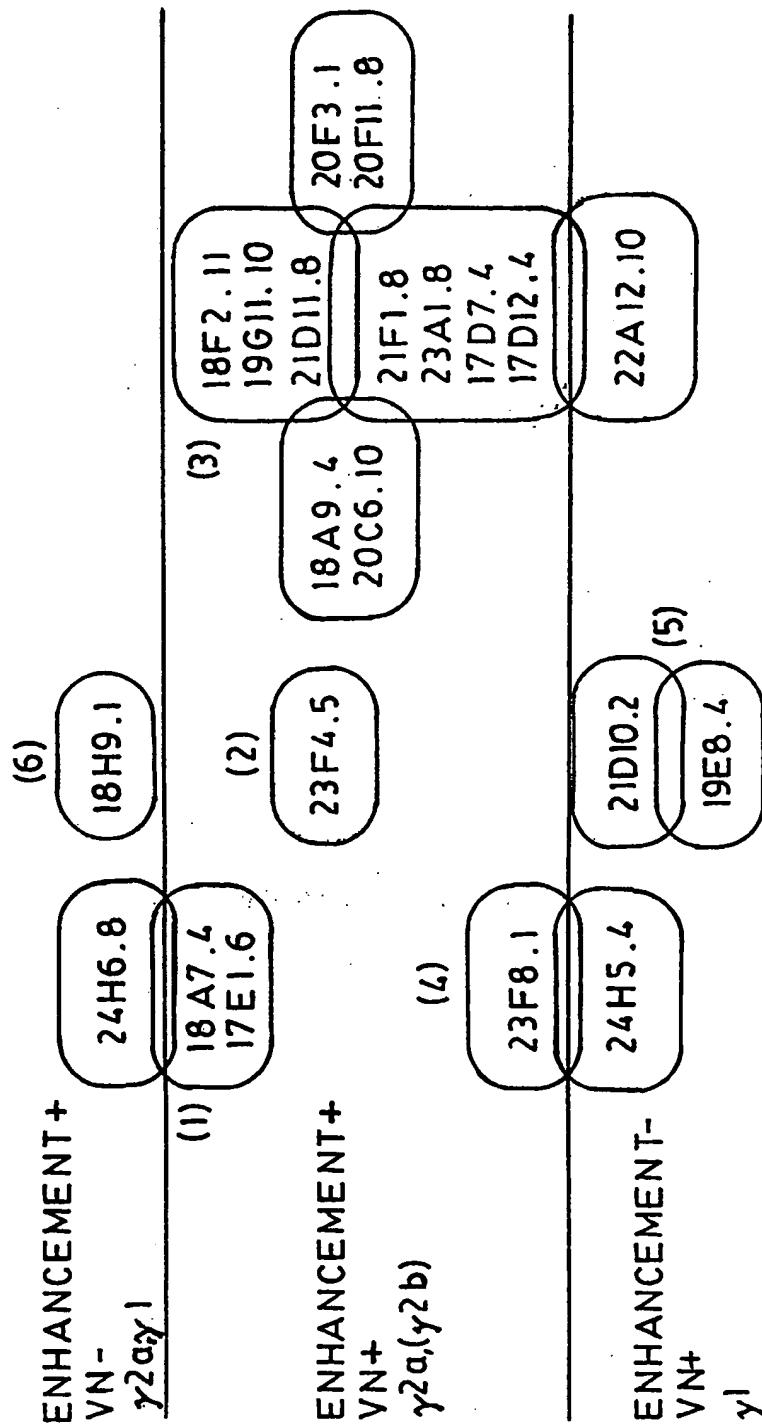


FIG. 4B

5/5

FIG.5

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/10634

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) : Please See Extra Sheet.

US CL : 530/388.3; 424/147.1; 435/240.27

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/388.3; 424/147.1; 435/240.27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, SCISEARCH, LIFE SCIENCE, CAB ABSTRACTS, BIOSIS, MEDLINE, PASCAL, EMBASE, CURRENT CONTENTS, AGRICOLA, CHEMSEARCH.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Virology, Volume 66, Number 2, issued February 1992, C. W. Olsen et al., "Monoclonal Antibodies to the Spike Protein of Feline Infectious Peritonitis Virus Mediate Antibody-Dependent Enhancement of Infection of Feline Macrophages", pages 956-965, see entire document, especially Table 1.	1-18
X	Journal of Clinical Microbiology, Volume 22, Number 3, issued September 1985, Fiscus et al., "Competitive Enzyme Immunoassays for the Rapid Detection of Antibodies to Feline Infectious Peritonitis Virus Polypeptides", pages 395-401, see the entire document.	1-2 and 18

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Virology, Volume 66, Number 11, issued November 1992, Corapi et al., "Monoclonal Antibody Analysis of Neutralization and Antibody-Dependent Enhancement of Feline Infectious Peritonitis Virus", pages 6695-6705, see entire document.	1-18
X	Archives of Virology, Volume 120, issued 1991, Hohdatsu et al., "A Study on the Mechanism of Antibody-Dependent Enhancement of Feline Infectious Peritonitis Virus Infection in Feline Macrophages by Monoclonal Antibodies", pages 207-217, see entire document.	1-9 and 18
X	Microbiology Immunology, Volume 37, Number 6, issued 1993, Hohdatsu et al., "Enhancement and Neutralization of Feline Infectious Peritonitis Virus Infection in Feline Macrophages by Neutralizing Monoclonal Antibodies Recognizing Different Epitopes", pages 499-504, see entire document.	1-9 and 18
Y	Veterinary Microbiology, Volume 37, issued 1993, L. J. Saif, "Coronavirus Immunogens", pages 285-297, see entire document.	19-21

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/10634

## A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C07K 16/08, 16/18; A61K 39/42; C12N 5/12

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